January 1992

An examination and correction of plant tissue culture basal medium formulations

Henry R. Owen
Eastern Illinois University, hrowen@eiu.edu

A. Raymond Miller
Ohio State University

An examination and correction of plant tissue culture basal medium formulations

Henry R. Owen & A. Raymond Miller

Abstract

The inorganic formulations of fourteen common plant tissue culture basal media were examined from the primary literature. Inaccuracies and errors were found for molecular formulae, chemical hydrations, and molar equivalences for iron/EDTA complexation. A comparison with published basal medium formulations from six commercial suppliers uncovered additional inaccuracies, modifications, and errors, thereby emphasizing the need for investigators to examine and describe medium formulations precisely in future publications.

The purpose of this paper is to draw attention to a number of inaccuracies and errors which have appeared in several widely-used plant tissue culture basal medium formulations. Errors have occurred in the primary literature and these errors have been perpetuated in secondary and tertiary citations. To compound the problem, different formulations have been published for the same basal medium, even by the same investigator or their co-workers (White 1943; White 1963). Some of these errors have been corrected (Singh & Krikorian 1980, 1981); however, more often when corrections have been made, they have been made without noting the original error. Additionally, inconsistencies exist in popular commercial preparations for several common basal media, over and above those found in the primary literature. For general plant developmental responses in vitro (i.e. organogenesis), most plant tissues are very tolerant of minor variations in medium components. However, as plant cell and tissue culture methodologies have become more defined (i.e. microspore and protoplast culture), it has been shown that minor variations in medium composition can determine the success or failure of certain protocols.

We have provided in Table 1 the corrected inorganic composition of eight common plant tissue culture basal media and indicated the compounds for which errors have occurred. The organic constituents of media (including carbohydrates, complex addenda, vitamins, and growth regulators) are usually modified to suit a particular species, explant, or desired developmental or morphogenic pathway and, therefore, are not included.

One inaccuracy which has been perpetuated in many formulations is in the exact hydration and molar equivalence of iron and its chelating agent. As noted by Singh & Krikorian (1980), 0.1 mM $FeNa_2$-EDTA requires 27.8 mg 1^{-1} $FeS0_4 \cdot 7H_2 0$ and 37.2mgl^{-1} of dehydrated Na_2-EDTA (or 33.6 mg l^{-1} of anhydrous Na_2-EDTA). Many basal media that contain chelated iron, however, incorrectly list 37.3 mg 1^{-1} of anhydrous Na_2-EDTA in their formulations. These excesses of chelating agent, although small, may be influencing micronutrient availabilities. In addition to those media listed in Table 1 (all but White's media), inaccuracies for iron/EDT A exist in other basal medium formulations (Eriksson 1965; Schenk & Hildebrandt 1972; Gresshoff & Doy 1974).

Some errors were fairly easy to detect and correct, based on molecular formula, or natural occurrence or commercial availability of particular hydrates. For example, $NaS0_4$ (White 1963) should be Na_2S0_4, based on molecular formula; $ZnS0_4 \cdot 4H_20$ (Murashige & Skoog 1962) should be $ZnS0_4 \cdot 7H_20$, based on the natural occurrence of hydrates; and $CuS0_4$ (Gamborg et al. 1968) should be $CuS0_4 \cdot 5H_2 0$, based on commercial availability. These last two errors have been perpetuated in other basal medium formulations (Nagata & Takebe 1971; Gresshoff & Doy 1974, respectively).

When several forms of a compound are readily available commercially, such as different hydrates of calcium chloride and manganese sulfate, it is more difficult to detect typographical errors. In these instances, errors may be deduced by examining molar concentrations and comparing a particular formula to other papers by the same investigator. Using this method, we reasoned that Woody Plant Medium (Lloyd & McCown 1980) should contain 22.3 mg $I\sim_1$ $MnS0_4 \cdot 4H_2 0$ (which equals 100 JLM), rather than 22.3 mg

1-1 of the monohydrate (which equals 132 JLM), and also because the tetrahydrate is the compound cited in a later paper by McCown (Smith & McCown, 1982/83). By the same type of reasoning, however, we question if the 166mgl~1 $CaCI_2$ $\cdot 2H_2$ 0 (1.13mM) for N6 medium (Chu 1975; Chu et al. 1978) should actually be the anhydrous form at 1.5 mM. This concentration (1.5 mM) is equal to the calcium concentration of Nitsch & Nitsch medium and one half the concentration of Murashige & Skoog medium. Blaydes (1966) did not list hydration values for calcium nitrate, magnesium sulfate, zinc sulfate, or manganese sulfate and did not give molar concentrations, thus making his medium uncertain as to its precise formulation.

Table 1. Corrected inorganic components ($mg\,l^{-1}$) of eight plant tissue culture basal media.[a]

	W43	W63	MS	B5	NN	N6	AN	WP
NH_4NO_3			1650		720		400	400
$(NH_4)_2SO_4$				134		463		
$MgSO_4 \cdot 7H_2O$	737[c]	720	370	246[i]	185	185	370	370
KCl	65	65						
KNO_3	80	80	1900	2528[j]	950	2830	480	
KH_2PO_4			170		68	400		170
K_2SO_4								990
$NaH_2PO_4 \cdot H_2O$	19[d]	19[d]		150			380	
Na_2SO_4	200	200[e]						
$CaCl_2$					166			
$CaCl_2 \cdot 2H_2O$			440	150		166	440	96
$Ca(NO_3)_2 \cdot 4H_2O$	288[c]	300						556
Na_2-EDTA $\cdot 2H_2O$[b]			37.2[g]	37.2[gk]	37.2[g]	37.2[g]	74.5[p]	37.2[g]
$FeSO_4 \cdot 7H_2O$			27.8	27.8[k]	27.8[n]	27.8	55.7	27.8
$Fe_2(SO_4)_3$	2.5	2.5						
H_3BO_3	1.5	1.5	6.2	3	10	1.6	6.2	6.2
$CoCl_2 \cdot 6H_2O$			0.025	0.025			0.025[q]	
$CuSO_4 \cdot 5H_2O$		0.001[f]	0.025	0.025[m]	0.025		0.025[r]	0.25
$MnSO_4 \cdot H_2O$				10			16.9	
$MnSO_4 \cdot 4H_2O$	6.65[c]	7	22.3		25	4.4		22.3[u]
MoO_3		0.0001[f]						
$Na_2MoO_4 \cdot 2H_2O$			0.25	0.25	0.25		0.25[s]	0.25
KI	0.75	0.75	0.83	0.75		0.8	0.30[t]	
$ZnSO_4 \cdot 7H_2O$	2.67[c]	3	8.6[h]	2	10	1.5	8.6	8.6

[a] based on original citations, except where noted. The reader is advised to examine commercial preparations and secondary citations since many inconsistencies have occurred. W43 = White (1943); W63 = White (1963); MS = Murashige & Skoog (1962); B5 = Gamborg et al. (1968); NN = Nitsch & Nitsch (1969); N6 = Chu (1975), Chu et al. (1978); AN = Anderson (1980); WP = Woody Plant Medium (Lloyd & McCown 1980).
[b] dihydrate used in all medium formulations
[c] originally stated in anhydrous form (see Singh & Krikorian 1981)
[d] originally printed as 16.5 $mg\,l^{-1}$ (see Singh & Krikorian 1981)
[e] originally printed as $NaSO_4$
[f] as added by Boll & Street (1951)
[g] originally stated as 37.3 $mg\,l^{-1}$ of anhydrous form (see Singh & Krikorian 1980)
[h] originally printed as $ZnSO_4 \cdot 4H_2O$
[i] stated as 250 $mg\,l^{-1}$ in Gamborg et al. 1976
[j] stated as 2500 $mg\,l^{-1}$ in Gamborg et al. 1976
[k] replaced with MS iron formulation (see Gamborg et al. 1976)
[m] originally printed as $CuSO_4$ (see Gamborg et al. 1976)
[n] originally printed as 0.557 $g\,l^{-1}$ for stock solution
[p] originally printed as 74.5 $mg\,l^{-1}$ of anhydrous form (see Singh & Krikorian 1980)
[q] originally printed as $CaCl_2 \cdot 6H_2O$ (see Anderson 1983)
[r] originally printed as $CaSO_4 \cdot 5H_2O$ (see Anderson 1983)
[s] originally printed as $Ma_2MoO_4 \cdot 2H_2O$
[t] originally printed as Kl
[u] originally printed as $MnSO_4 \cdot H_2O$ (see Smith & McCown 1982/83)

Typographical errors which substitute one element for another may also be difficult to detect. By comparing Anderson's original publication of his shoot multiplication medium (Anderson 1980) to a later publication (Anderson 1983) we concluded that substitutional errors existed (calcium for copper and calcium for cobalt) in his original publication.

Inconsistencies also were detected between original published formulations and formulations of commercially available preparations of commonly used basal media. For most of the basal media listed in Table 1, as well as other media (Gresshoff & Doy 1974; Knudson 1946; Vacin & Went 1949), at least one commercial supplier of those examined (Carolina Biological Supply Co, Connecticut Valley Bioi. Supply Co, Gibco 149 BRL, ICN Biomedicals Inc, Research Organics Inc, and Sigma Chemical Co) presents incorrect formulations in their catalog or other technical information. For example, for B5 medium, one supplier lists four times the published amount of calcium and also adds ammonium nitrate to the formulation. For MS medium, another supplier lists anhydrous $CaCl_2$ and $MgSO_4$ in one of its formulations, but in amounts corresponding to the hydrates listed in the original publication. Thus, investigators should examine original papers carefully and compare them with commercial formulations.

It is hoped that these examples will impress upon investigators the need to detail medium formulations precisely, indicate all modifications to formulations cited, and identify any commercial preparations used.

Acknowledgements

Supported in part by State and Federal funds appropriated to The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, Ohio. Manuscript No. 143-91. This work is dedicated to the memory of Carroll Gene Manuel II.

References

Anderson WC (1980) Tissue culture propagation of red and= black raspberries, Rubus idaeus and R. occidentalis. Acta Hort. 112: 13-20

Anderson WC (1983) Micropropagation of filberts, Corylus avellana. Comb. Proc. Intern. Plant Prop. Soc. 33: 132-137 (Pub!. 1984)

Blaydes DF (1966) Interaction of kinetin and various inhibitors in the growth of soybean tissue. Physiol. Plant. 19: 748-753

Boll WG & Street HE (1951) Studies on the growth of excised roots. I. The stimulatory effect of molybdenum and copper on the growth of excised tomato roots. New Phytol. 50: 52-75

Chu C-C (1978) The N_6 medium and its applications to anther culture of cereal crops. In: Proc. Symp. on Plant Tiss. Cult. Science Press, Peking, pp 43-50

Chu C-C, Wang C-C, Sun C-S, Hsu C, Yin K-C, Chu C-Y & Bi F-Y (1975) Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Scientia Sinica 18: 659-668

Eriksson T (1965) Studies on the growth requirements and growth measurements of cell cultures of Haplopappus gracilis. Physiol. Plant. 18: 976-993

Gamborg OL, Miller RA & Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Experimental Cell Research 50: 151-158

Gamborg OL, Murashige T, Thorpe TA & Vasil IK (1976) Plant Tissue Culture Media. In Vitro 12: 473-478

Gresshoff PM & Doy CH (1974) Derivation of a haploid cell line from Vitis vinifera and the importance of the stage of meiotic development of anthers for haploid culture of this and other genera. Z. Pflanzenphysiol. 73: 132-141

Knudson L (1946) A new nutrient solution for the germination of orchid seed. Amer. Orchid Soc. Bull. 15: 214-217

Lloyd G & McCown B (1980) Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. Intern. Plant Prop. Soc. Proc. 30:421-427 (Publ 1981)

Murashige T & Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497

Nagata T & Takebe I (1971) Plating of isolated tobacco mesophyll protoplasts on agar medium. Planta 99: 12-20

Nitsch JP & Nitsch C (1969) Haploid plants from pollen grains. Science 163: 85-87

Schenk RU & Hildebrandt AC (1972) Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. 50: 199-204

Singh M & Krikorian AD (1980) Chelated iron in culture media. Ann. Bot. 46: 807-809

Singh M & Krikorian AD (1981) White's standard nutrient solution. Ann. Bot. 47: 133-139

Smith MAL & McCown BH (1982/83) A comparison of Source tissue for protoplast isolation from three woody plant species. Plant Sci. Letters 28: 149-156

Vacin EF & Went FW (1949) Some pH changes in nutrient solutions. Botanical Gaz. 110: 605-613

White PR (1943) A Handbook of Plant Tissue Culture. The Jaques Cattell Press, Lancaster, Penn, 277 p

White PR (1963) The cultivation of animal and plant cells. Ronald Press Co, New York, 228 p